Journal of Medical Virology 55:177–183 (1998)

A Comparison of PCR With Virus Isolation and Direct Antigen Detection for Diagnosis and Typing of Genital Herpes

M.J. Slomka,[1]* L. Emery,[2] P.E. Munday,[3] M. Moulsdale,[2] and D.W.G. Brown[1]

[1]*Enteric and Respiratory Virus Laboratory, Central Public Health Laboratory, London, United Kingdom*
[2]*Department of Microbiology, Watford General Hospital, Watford, Herts, United Kingdom*
[3]*Department of Genitourinary Medicine, Watford General Hospital, Watford, Herts, United Kingdom*

Patients attending the genitourinary medicine clinic at Watford General Hospital, UK, were examined for clinical signs of genital herpes infection. Genital swabs were taken from 194 patients (126 female, 68 male) who presented with genital ulceration or symptoms which were suggestive of genital herpes infection. Swabs from these patients were tested by three methods: (i) Detection of herpes simplex virus (HSV) antigen by direct HSV enzyme immunoassay (EIA), (ii) HSV isolation in Vero cell culture and (iii) HSV polymerase chain reaction (PCR). HSV was detected in 76 patients (39%) by EIA, in 93 (48%) by isolation in cell culture, and in 115 (59%) by PCR. Isolation by cell culture has been considered as the ''gold standard'' for the detection of HSV in genital lesions, but in this study HSV PCR was significantly more sensitive. Comparison of the three methods was as follows: Cell culture vs. PCR: Sensitivity 93/115 (80.9%), Specificity 79/79 (100%). HSV EIA vs. PCR: Sensitivity 75/115 (65.2%), Specificity 78/79 (98.7%). HSV EIA vs. Cell culture: Sensitivity 75/93 (80.7%), Specificity 100/101 (99%). EIA was less effective in detecting HSV among recurrent than among first episode infections, in comparison to culture or HSV PCR. This is the first comparison of HSV PCR with two other routine diagnostic methods for confirming genital herpes infection in a symptomatic population. The infecting HSV type was identified by restriction digestion of 108 HSV amplicons: HSV-1: 37/108 (34%), HSV-2: 71/108 (66%). In this population HSV-1 causes a significant proportion of genital herpes cases, and HSV-1 genital infection was detected in significantly more first episode infections (40.3%) than among recurrent infections (22.2%). ***J. Med. Virol. 55:177–183, 1998.***

KEY WORDS: genital herpes; PCR; virus isolation in cell culture; HSV typing; first episode infection; recurrent infection

INTRODUCTION

Genital herpes is a sexually transmitted infection which is classically caused by herpes simplex virus type 2 (HSV-2), although in recent years a proportion of genital herpes cases have been found to be caused by herpes simplex virus type 1 (HSV-1) [Mertz, 1993; Whitley and Gnann, 1993], particularly in the UK [Barton et al., 1982; Lavery et al., 1986; Ross et al., 1993; Tayal and Pattman, 1994]. Primary genital HSV infection is followed by latent infection in the sacral ganglia, this being the source of periodic recurrent infections. The majority of genital HSV infections are subclinical, and asymptomatic shedding is an important source of transmission of HSV infection to sexual partners [Mertz, 1993; Slomka, 1996].

Among the laboratory methods available for confirmation of HSV infection, virus isolation in tissue culture has been shown to be a more sensitive technique than direct antigen enzyme immunoassay (HSV EIA) [Verano and Michalski, 1995]. However, virus isolation is slower and dependent on access to tissue culture facilities. In recent years, the highly sensitive polymerase chain reaction (PCR) has been used for diagnosis of HSV infections in the central nervous system (Lakeman et al., 1995), although it has not yet been accepted for the routine diagnosis of genital herpes (Ashley, 1993). The aim of the present study was to compare the sensitivity and specificity of cell culture and HSV EIA with HSV PCR in a population of patients attending the genitourinary medicine (GUM) clinic at Watford General Hospital (UK). Laboratory diagnosis is important for the confirmation of genital herpes, particularly as its clinical spectrum is diverse and there are other

The current address of M.J. Slomka is Murex Biotech Ltd, Central Road, Dartford, Kent DA1 5LR, United Kingdom.

The current address of L. Emery is Dept of Clinical Microbiology, Cecil Flemming House, University College Hospital, Grafton Way, London WC1E 6BD, United Kingdom.

*Correspondence to: M.J. Slomka, Murex Biotech Ltd, Central Road, Dartford, Kent DA1 5LR, United Kingdom.

Accepted 3 July 1997

microbiological and dermatological causes of genital ulcerative disease [Corey, 1994]. Typing of laboratory confirmed HSV isolates may be helpful for counselling patients and their sexual partners, as well as providing epidemiological information pertaining to sexually-active high-risk populations. In the present study, HSV typing was carried out by monoclonal antibody immune fluorescence (Mab IF) on culture-positive isolates and by restriction enzyme digestion of PCR products.

MATERIALS AND METHODS

Patient Population

The study was approved by the ethical committee at Watford General Hospital, UK. Genito-urinary medicine clinic patients (n = 194; 68 male, 126 female) were enrolled if they had symptoms or signs suggestive of genital herpes infection. Clinical illness was recorded as either a first episode or recurrent infection, and genital swabs obtained between 1 and 14 days after onset of symptoms.

Genital Swabs

Two swabs were obtained from each patient, a "dry" swab and a "wet" swab soaked in 0.5–0.7 ml virus transport medium (Virocult; Medical Wire and Equipment, Cleveland, OH). Swabbing of the genitalia was randomised such that for 50% of patients the dry swab was taken first, while for the remaining 50% the wet swab was taken first.

Direct HSV EIA

The dry swab was tested for HSV antigen by Wellcozyme HSV ELISA (Murex). Swabs were stored at 4°C for up to 3 days prior to HSV EIA testing, within the recommended period of 7 days as described in the manufacturer's protocol. Borderline EIA results were accepted as positive for HSV antigen.

HSV Isolation in Cell Culture and Mab IF Typing

Vero cells were grown to near confluence at 37°C in 25 cm^2 tissue culture flats containing Eagle's Minimal Essential Medium (Life Technologies) containing 5% fetal calf serum (FCS). Wet swabs were taken directly from the clinic to the laboratory and approximately 0.3 ml Virocult swab specimen was inoculated on to the Vero cell monolayer for continued incubation. Cultures without cytopathic effect (cpe) were discarded after 10 days and reported as HSV negative. For the majority of cpe yielding isolates, Vero cells were harvested for Mab IF HSV typing. The Micro Trak HSV-1 and HSV-2 culture/identification typing test (Syva) was used. This utilised fluoroscein-labelled Mabs which react with HSV-1 glycoprotein C, HSV-2 ribonucleotide reductase and HSV-2 glycoprotein B.

DNA Extraction From Virocult

After inoculation into tissue culture flats, the remaining Virocult was stored frozen at –30°C until required for PCR. Two hundred microlitres thawed Virocult was extracted by addition of 10 μl salt concentrate which gave final concentrations of 10 mM Tris-HCl (pH 8.0), 1 mM EDTA, 0.25% SDS. Proteinase K (Boehringer) was added to a final concentration of 500 μg/ml and incubated at 56°C for 1 hr. The digest was extracted once with an equal volume of phenol: chloroform (1:1), and once with an equal volume chloroform: isoamylalcohol (24:1) followed by addition of 20 μl 3 M sodium acetate (pH 5.0) and 5 μg glycogen. Five hundred microlitres cold ethanol was added to precipitate nucleic acid overnight at –25°C. After pelleting in an Eppendorf centrifuge, the pellets were dried at 56°C for 15–20 mins and resuspended in 100 μl sterile distilled water.

HSV PCR

HSV PCR primers were selected which amplify a 330 bp sequence within the HSV DNA polymerase gene [Kimura et al., 1991]. Optimal PCR conditions included each primer at 0.2 μM in 100 μl final reaction volume, 200 μM each dNTP, 10 mM Tris-HCl (pH 8.8), 75 mM potasium chloride and 1.5 mM magnesium chloride. A GEM100 wax bead (Perkin Elmer/Applied Biosystems) was included to effect a "hot start" PCR [Chou et al., 1992], and 25 μl extracted DNA added to each PCR tube. After initial denaturation at 96°C for 30 sec, cycling continued immediately on a Trio Thermoblock at 96°C (1 min), 67°C (1 min), 72°C (1.5 min) (×35) with the final 72°C extension being prolonged to 5 min. When extracting and amplifying clinical specimens, 200 μl of sterile distilled water was extracted in parallel for PCR as a negative control. Positive controls were also extracted for PCR.

Detection of HSV Amplicons

Twenty five microlitres PCR product were electrophoresed through a 2% agarose gel and stained in ethidium bromide for visualisation on a UV source. Amplification products which were apparently negative by ethidium bromide staining were re-tested by dot-blot hybridisation as follows: 10 μl PCR product was added to 40 μl TAE followed by 50 μl 1 M NaOH and incubated at room temperature for 10 mins. The total 100 μl volume was applied to a prewetted (×2 SSC) positively charged nylon membrane (Boehringer) using a vacuum manifold (Bio Rad). One hundred microlitres 0.5 M Tris-HCl (pH 7.5) 1.5 M sodium chloride was added to each well to neutralize, the membrane dismantled, dried and baked at 120°C for 30 mins to fix the DNA. Prehybridisation of the membrane was for 2 hours at 42°C in 10ml prehybridisation fluid (×5 SSC, 0.3% blocking buffer (Boehringer), 0.1% sarcosyl, 0.01% SDS). An oligonucleotide probe (23 nucleotides) specific to the 330 bp HSV amplicon [Kimura et al., 1991] was 3′ end labelled with digoxygenin tagged ddATP using terminal transferase as described by the manufacturer (Boehringer), purified, and added to 5 ml fresh prehybridisation fluid to a concentration of 4 p mol probe per ml. Hybridisation continued overnight at 42°C followed by washing in (×2) SSC 0.1% SDS (twice) and (0.1×) SSC 0.1% SDS (twice) (each wash

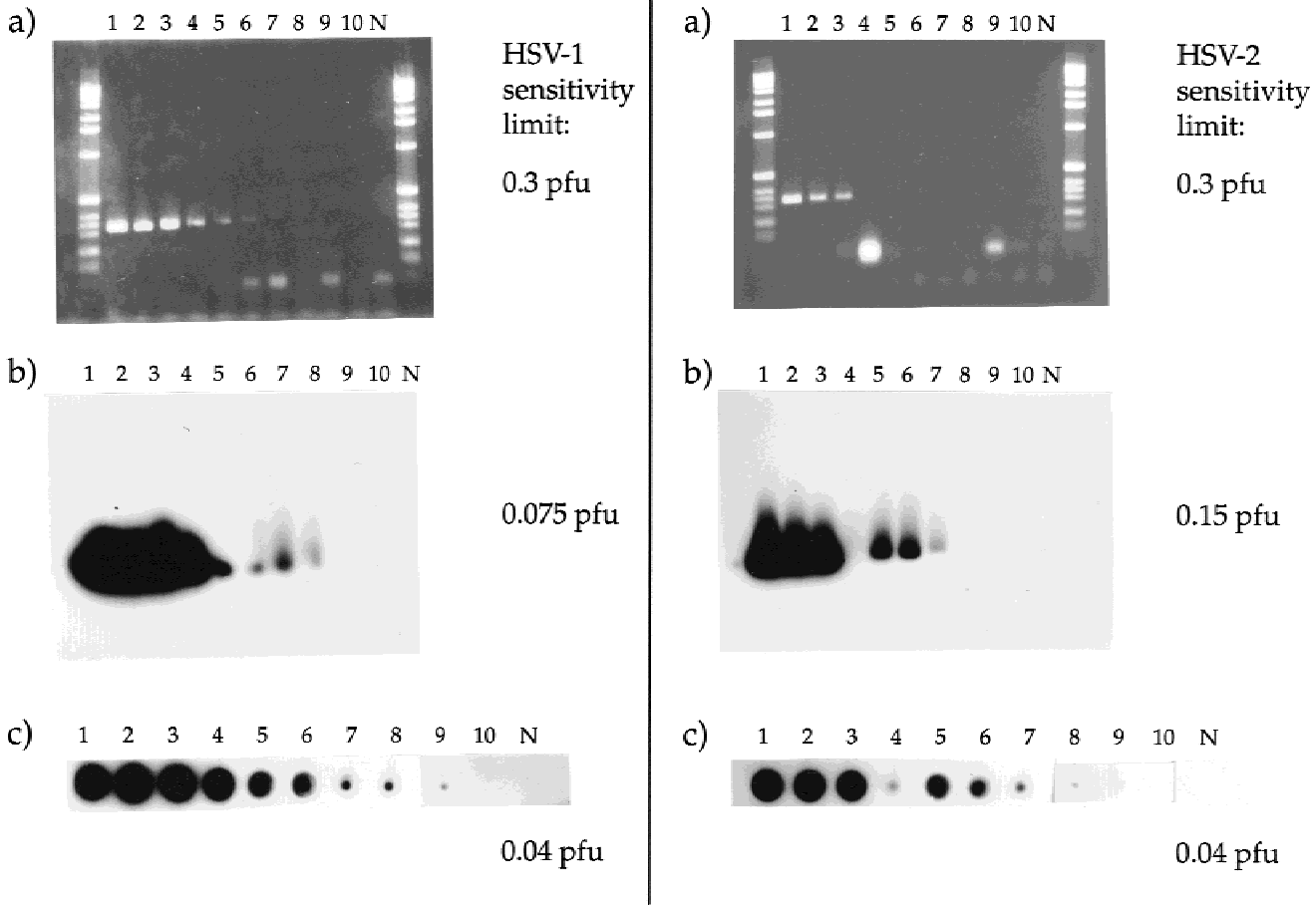

Fig. 1. Sensitivity of HSV PCR determined by amplifying dilutions of HSV-1 (left-hand panels) and HSV-2 (right-hand panels). Two-fold dilutions series were prepared in 200 μl Virocult, extracted, resuspended and amplified as described in the text. Assuming 100% recovery by extraction, target quantity ranged from 10 pfu to 0.02 pfu HSV (tracks 1 to 10 respectively). Extracted Virocult which did not contain diluted HSV served as a negative control (N). HSV amplicons were detected by **(a)** ethidium bromide staining of agarose gels, **(b)** Southern blotting with hybridisation, and **(c)** dot blot hybridisation.

being at 42°C for 15 min), and this was followed by immune detection of hybridised digoxygenin-labelled probe using anti-digoxygenin alkaline phosphatase and CSPD chemiluminescence (Boehringer), the membrane being exposed to Hyperfilm-ECL (Amersham) for between 30 secs and 20 min. PCR detection of HSV was performed blind with regard to the EIA and virus isolation results.

Restriction Typing of HSV Amplicons

In cases where extracted Virocult yielded a strong HSV amplicon by ethidium bromide staining, 5 μl volumes of PCR product were directly typed by restriction digestion with *Bgl* II and *Xho* I [Ando et al. 1993]. For fainter PCR products and those which were positive only by dot-blot hybridisation, amplicons were fractionated through Chroma Spin-100 TE columns (Clontech) which included glycogen according to the supplier's protocol. The eluate was concentrated by ethanol precipitation as described earlier, and the pellet resuspended in 20 μl sterile distilled water for restriction typing. Restricted amplicons were electrophoresed through a 1.7% agarose gel which was stained in ethidium bromide. Where gels included apparently invisible amplicons, Southern blotting was performed on positively charged nylon membranes (Boehringer) using 0.4 M NaOH for transfer followed by hybridisation with the oligonucleotide probe as described for dot-blot hybridisation. All restriction typing experiments were carried out blind with regard to the Mab IF results.

RESULTS

Sensitivity of the HSV PCR

Experiments were conducted where 2-fold dilutions of HSV-1 SC16 and HSV-2 186 strains of known titre were extracted and amplified by the HSV PCR. The least quantity of HSV detected by ethidium bromide staining of agarose gels was 0.3 pfu of either HSV-1 or HSV-2 (discernible on original gel photograph, Fig. 1). Southern blotting and oligonucleotide probe hybridisation increased the sensitivity of the PCR to 0.075 and 0.15 pfu for HSV-1 and -2 respectively (Fig. 1). Dot-

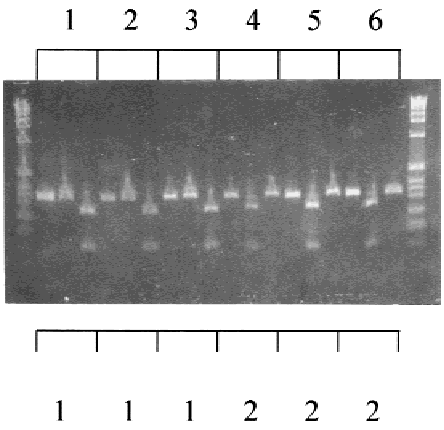

Fig. 2. Restriction digestion of HSV amplicons obtained from six clinical specimens. Each set of three tracks contained undigested amplicon, *Bgl* II and *Xho* I incubated amplicon, left to right respectively. The HSV typing result is indicated at the foot of the figure.

blotting increased the HSV PCR sensitivity to 0.04 pfu for each type (visible on original membranes, Fig. 1).

Detection and Typing of HSV From Clinical Specimens by PCR

A representative gel showing HSV amplicons from clinical specimens is shown (Fig. 2), and also demonstrates how restriction endonuclease digestion served to type the HSV amplicons. All amplicons which were apparently HSV negative by ethidium bromide staining of agarose gels were retested by dot blot hybridisation. The importance of this diagnostic strategy is illustrated by dilution 4 (1.25 pfu HSV-2) where ethidium bromide staining and Southern blotting failed to reveal a clear HSV amplicon (Fig. 1a,b), but dot blot hybridisation revealed a positive HSV signal (Fig. 1c).

Detection of HSV by HSV EIA, Cell Culture and HSV PCR

Out of 194 patients who were investigated, HSV was detected in 76 (39%) by EIA, in 93 (48%) by cell culture and 115 (59%) by PCR (Table I). Five of the 115 PCR positives were identified by dot blot hybridisation after failing to amplify a visible product by ethidium bromide staining. Swabs from male and female genitalia were obtained from the site of the lesions, including the penis, urethra, cervix or vulva as appropriate. One patient presented with simultaneous herpetiform lesions on the penis, mouth and eye: All three swabs were positive for HSV in the three diagnostic procedures employed, while typing by Mab IF and PCR showed the three sites to be infected with HSV-1. An additional patient who did not present with genital lesions (thereby excluded from Table I) was found to have HSV ocular infection (positive by all three methods) which was identified as HSV-2 by Mab IF and PCR.

TABLE I. Sensitivities and Specificities for Comparisons Between a) Cell Culture and PCR, b) HSV EIA and HSV PCR, c) HSV EIA and Cell Culture

a)

		HSV PCR +	HSV PCR −	
Cell culture	+	93	0	93
	−	22[a]	79	101
		115	79	194

Sensitivity of cell culture vs. PCR: 93/115 = 80.9%
Specificity of cell culture vs. PCR: 79/79 = 100%

b)

		HSV PCR +	HSV PCR −	
HSV EIA	+	75[b]	1	76
	−	40[a]	78	118
		115	79	194

Sensitivity of EIA vs. PCR: 75/115 = 65.2%
Specificity of EIA vs. PCR: 78/79 = 98.7%

c)

		Cell culture +	Cell culture −	
HSV EIA	+	75[b]	1	76
	−	18	100	118
		93	101	194

Sensitivity of EIA vs. cell culture: 75/93 = 80.7%
Specificity of EIA vs. cell culture: 100/101 = 99%

[a]Includes 5 specimens where HSV amplicons were negative by ethidum bromide staining but positive by dot-blot hybridisation.
[b]Includes 5 specimens which were borderline positive by HSV EIA.

Comparison of the Three Diagnostic Methods (Sensitivity and Specificity)

Table I shows pairwise comparisons of testing by HSV EIA, cell culture and HSV PCR. Seventy five swabs were HSV positive by all three methods, while a further 18 and 40 swabs were positive when tested by cell culture and PCR, respectively. The 75 positive EIA specimens included 5 swabs which a gave a borderline result as per the manufacturer's definition of cut-off values (Table I). One hundred and ten of the HSV PCR positive swabs were positive by ethidium bromide staining of agarose gels (Fig. 2), while a further 5 amplicons were negative by gel staining but HSV positive by dot blot hybridisation (Table I). In addition one genital swab was HSV positive by EIA but negative by both cell culture and HSV PCR. Calculations demonstrated increasing sensitivity in the order HSV EIA, cell culture and HSV PCR, while the specificities of all three assays were very high (Table I). EIA detected a lower proportion of genital herpes cases among recurrences than among first episodes, while culture and/or HSV PCR detected a greater proportion among the former (Table II). Sensitivity of the HSV EIA was quantified further where two-fold dilution series of titrated HSV-1 SC16 and HSV-2 186 were tested, giving a detection limit of 75 pfu HSV (both types) per 150 μl "Wellco-

TABLE II. Numbers of Genital HSV Isolates (First Episodes and Recurrences) Which Were Detected by Different Combinations of the Three HSV Diagnostic Methods

	EIA, culture and PCR	Culture and PCR	PCR only
First episodes (n = 75)	55 (73.3%)	8 (12%)	12 (14.7%)
Recurrences (n = 40)	20 (50%)	10 (25%)	10 (25%)

Brackets indicate percentages among first episodes and recurrences. EIA positives include 5 borderline results, while "PCR only" positives include 5 specimens which were negative by ethidium bromide staining but positive by dot-blot hybridisation (3/12 first episodes and 2/10 recurrences).

zyme HSV extraction buffer" which corresponds to 500 pfu HSV per swab, in accordance with the manufacturer's instructions and definition of the cut-off value.

HSV Typing by Mab IF and Restriction Digestion of HSV Amplicons

Mab IF typing of HSV isolated by cell culture in 83 HSV positive swabs demonstrated 28 (34%) to be HSV-1 and 55 (66%) to be HSV-2 (Table III). Restriction digestion of amplicons from 108 HSV positive patients revealed 37 (34%) to be HSV-1 and 71 (66%) to be HSV-2 (Table III). There were no contradictory typing results by the two methods. Seven PCR positive swabs were not typed by restriction: four of these were HSV PCR positive only after dot-blot hybridisation but negative by staining of the agarose gel, and were below or dropped below the detection limit of Southern blotting due to likely sample loss during Chromaspin purification and ethanol precipitation. Three swabs yielded faint 330 bp amplicons by ethidium bromide staining and were confirmed by dot blot hybridisation, but subsequent amplicon loss resulted in failure to be typed.

HSV Typing: First Episode and Recurrent Genital Infection

The 115 PCR positives included 75 and 40 first episode and recurrent HSV infections respectively. Among the 72 typed swabs from first episodes, 29 (40.3%) and 43 (59.7%) were due to HSV-1 and HSV-2 infection respectively. For the 36 typed recurrences, 8 (22.2%) and 28 (77.8%) were due to HSV-1 and HSV-2 infection respectively.

TABLE III. HSV Typing of Genital Swabs by Mab IF and Restriction Typing of HSV Amplicons

	Mab IF (cell culture positive) (n = 83)	Restriction typing of HSV amplicons (n = 108)
HSV-1	28 (34%)	37 (34%)
HSV-2	55 (66%)	71 (66%)
Total	83 (100%)	108 (100%)

Ten swabs which were isolated by culture were not typed, while seven swabs which were PCR positive were not typed.

DISCUSSION

The design of this study involved minimal modification (i.e. two genital swabs obtained per patient) of the routine working practices of the Genito-urinary Medicine and Microbiology Departments at the hospital. It compared three diagnostic techniques for genital herpes, and served to demonstrate the potential for HSV PCR as regards sensitivity and HSV typing.

The direct antigen HSV EIA is an easy to use, rapid (approximately 5 hrs) technique for identifying HSV in genital swabs. It was shown to be highly specific, but its sensitivity was low in comparison with virus isolation and HSV PCR. Previous investigations have evaluated HSV EIAs from different manufacturers in comparison with virus isolation. For example, the "Herpchek" HSV EIA (DuPont) has featured in several evaluations, giving an average sensitivity of 91.1% (range: 62–100%), and an average specificity of 99.1% (range: 95.2–100%) [Verano and Michalski, 1995]. The Wellcozyme HSV EIA used in the present study had a sensitivity of 80.7% which fell within the broad sensitivity range described for Herpcheck and other commercial HSV EIAs. The low sensitivity of EIA was most apparent in recurrent disease (Table II).

In the present study there were 19 discrepant specimens for EIA and virus isolation (Table Ic), 18 of which were EIA negative/isolation positive. These 18 appeared to be negative due to the lack of sensitivity of the Wellcozyme EIA because all 18 were also HSV PCR positive. One specimen was EIA positive but isolation and HSV PCR negative. This could represent a false positive EIA result, however since EIA tests were performed with separate swabs from those used for isolation and PCR, it may represent a sampling error. While it is claimed that Herpchek HSV EIA can detect non-infectious virions [Cone et al., 1993], the possibility of false positives by EIA has been noted [Verano and Michalski, 1995].

The performance of virus isolation was clearly superior to HSV EIA in the present study, this being compatible with previous findings. Most isolation positive specimens (85) developed cpe within 48 hours of virus culture, but for 8 specimens virus culture was continued for up to 5 days to be scored as isolation positive. Minimal transit time between patient swabbing and inoculation of swab fluid into Vero cell culture considerably reduced the chance of infectivity loss which can occur during prolonged transport [Jensen and Johnson, 1994; Skinner et al., 1997]. However, not all genito-urinary medicine departments have immediate access to cell culture facilities.

The application of HSV PCR for genital herpes has been restricted [Hardy et al., 1990], but one large survey has demonstrated its greater sensitivity for HSV shedding among asymptomatic pregnant women [Cone et al., 1994]. A recent study of anti-viral therapy for genital herpes utilised HSV PCR where its greater sensitivity over virus isolation was noted [Diaz-Mitoma et al., 1996]. The present study is, to our knowledge, the first significant comparison of three different HSV di-

agnostic methods in a symptomatic sexually transmitted diseases population where the greater sensitivity of PCR was apparent. This was most noticeable for HSV recurrent infections, and the low sensitivity of EIA was most clear in this category of patient (Table II).

A further advantage of the HSV PCR is its ability to provide typing information. This may be important for patient/partner counselling in that HSV-1 genital herpes recurs less frequently than HSV-2 genital herpes, and genital HSV-1 infection appears to be a consequence of oro-genital contact [Lafferty et al., 1987]. In contrast, HSV-2 genital infection is frequently associated with multiple sexual partners (reviewed by Slomka, 1996). Typing of HSV amplicons revealed 34% of 108 PCR positives to be infections caused by HSV-1 and 66% to be HSV-2 infections. HSV-1 infection was more common among first episodes in comparison to recurrent infections, and may reflect the lower rate of clinical recurrence reported for genital HSV-1 infections [Benedetti et al., 1994]. Other studies which utilised Mab IF typing have similarly shown HSV-1 to cause a significant proportion of UK genital herpes cases [Barton et al., 1982; Lavery et al., 1986; Ross et al., 1993; Tayal and Pattman, 1994]. By contrast, a PCR study in France identified 57 positive genital swabs, 51 (89.5%) of which were due to HSV-2 and only 6 (10.5%) due to HSV-1 infection [Lucotte et al., 1995]. In this regard HSV PCR is a potentially rapid and sensitive means of providing clinical and epidemiological information.

Despite its advantages and potential clinical benefits, PCR remains a labour-intensive procedure where the processing of large numbers of specimens is involved, and immediate access to cell culture for HSV isolation may presently provide a more rapid result for a proportion of HSV positive swabs. Alternative extraction processes such as boiling the specimen, the use of Chelex and guanidinium hydrochloride/silica particle purification of nucleic acid were considered [Ochert et al., 1994], but none of these more rapid procedures was found to be consistent in yielding positive PCR results from reconstructed specimens which contained low HSV titres spiked into Virocult (data not shown). Potential difficulties with inhibitors in HSV amplification from genital specimens have been noted [Hardy et al., 1990], but extraction involving phenol and ethanol precipitation can minimise this problem [Cone et al., 1994; Orle et al., 1996]. Virus isolation, long regarded as the "gold standard", is generally more sensitive than HSV EIA but for certain specimens it can take up to several days to obtain a diagnostic result. As an alternative to blotting and hybridisation, other amplicon detection systems are available which can reduce the time to check specimens which are apparently PCR negative by ethidium bromide staining [Lazar, 1994]. HSV PCR for genital herpes has been used successfully in a clinical research setting [Orle et al., 1996], while a present diagnostic role is more likely for atypical clinical presentations and recurrent genital herpes cases as shown in the present study. A wider diagnostic role for HSV PCR in routine genito-urinary medicine practice would have to incorporate improvements in sample preparation and faster amplicon confirmation methods. Furthermore, cost considerations for PCR in comparison to cell culture would have to be examined, although it is hoped that continued development in PCR and related processes will make nucleic acid amplification an attractive technique for genito-urinary medicine patient management.

ACKNOWLEDGMENTS

The collection of clinical specimens by staff at the Watford General Hospital GUM Department is acknowledged. The authors also wish to thank Miss Bharati R. Purohit, Mrs. Inger Seth and Mrs. Lisa Snowden for typing the manuscript.

REFERENCES

Ando Y, Kimura H, Miwata H, Kudo T, Shibata M, Morishima T (1993): Quantitative analysis of herpes simplex virus DNA in cerebrospinal fluid of children with herpes simplex encephalitis. Journal of Medical Virology 41:170–173.

Ashley RL (1993): Laboratory techniques in the diagnosis of herpes simplex infection. Genitourinary Medicine 69:174–183.

Barton IG, Kinghorn GE, Najem S, Al-Omar LS, Potter CW (1982): Incidence of herpes simplex virus type 1 and 2 isolated in patients with herpes genitalis in Sheffield. British Journal of Venereal Disease 58:44–47.

Benedetti J, Corey L, Ashley R (1994): Recurrence rates in genital herpes after symptomatic first-episode infection. Annals of Internal Medicine 121:847–854.

Chou Q, Russell M, Birch DE, Raymond J, Bloch W (1992): Prevention of PCR mis-priming and primer dimerization improves low copy number amplifications. Nucleic Acids Research 20:1717–1723.

Cone RW, Swenson PD, Hobson AC, Remington M, Corey L (1993): Herpes simplex virus detection from genital lesions: A comparative study using antigen detection (HerpChek) and culture. Journal of Clinical Microbiology 31:1774–1776.

Cone RW, Hobson AC, Brown Z, Ashley R, Berry S, Winter C, Corey L (1994): Frequent detection of genital herpes simplex virus DNA by polymerase chain reaction among pregnant women. JAMA 272: 792–796.

Corey L (1994): The current trend in genital herpes. Progress in prevention. Sexually Transmitted Disease 21:s38–s44.

Diaz-Mitoma F, Ruben M, Sacks S, MacPherson P, Caissie G (1996): Detection of viral DNA to evaluate outcome of antiviral treatment of patients with recurrent genital herpes. Journal of Clinical Microbiology 34:657–663.

Hardy DA, Arvin AM, Yasukawa LL, Bronzan RN, Lewinsohn DM, Hensleigh PA, Prober CG (1990): Use of polymearse chain reaction for successful identification of asymptomatic genital infection with herpes simplex virus in pregnant women at delivery. Journal of Infectious Diseases 162:1031–1035.

Jensen C, Johnson FB (1994): Comparison of various transport media for viability maintenance of herpes simplex virus, respiratory syncytial virus, and adenovirus. Diagnostic Microbiology of Infectious Diseases 19:137–142.

Kimura H, Futamura M, Kito H, Ando T, Goto M, Kuzushima K, Shibata M, Morishima T (1991): Detection of viral DNA in neonatal herpes simplex virus infection: Frequent and prolonged presence in serum and cerebrospinal fluid. Journal of Infectious Diseases 164:289–293.

Lafferty WE, Coombs RW, Benedetti J, Critchlow C, Corey L. (1987): Recurrences after oral and genital herpes simplex virus infection. Influence of site of infection and viral type. New England Journal of Medicine 316:1444–1449.

Lakeman FD, Whitley RJ, Natl Institute of Allergy and Infectious Diseases Collaborative Antiviral Study Group (1995): Diagnosis of herpes simplex encephalitis: application of polymerase chain reaction to cerebrospinal fluid from brain-biopsied patients and correlation with disease. Journal of Infectious Diseases 171:857–863.

Lavery HA, Connolly JH, Russell JD (1986): Incidence of herpes genitalis in Northern Ireland in 1973–1983 and herpes simplex type 1 and 2 isolated in 1982–1984. Genitourinary Medicine 62:24–27.

Lazar JG (1994): Advanced methods in PCR product detection. PCR Methods and Applications 4:S1–S14.

Lucotte G, Bathelier C, Lespiaux V, Bali C, Champenois T (1995): Detection and genotyping of herpes simplex virus types 1 and 2 by polymerase chain reaction. Molecular and Cellular Probes 9:287–290.

Mertz GJ (1993): Epidemiology of genital herpes infections. Infectious Disease Clinics of North America 7:825–839.

Ochert A, Slomka MJ, Ellis J, Teo CG (1994): Use of chelex 100 in the extraction of viruses from diverse cell-free clinical samples for PCR. In Rolfs A, Weber-Rolfs I, Finckh U (eds): "Methods in DNA amplification". New York and London: Plenum Press, pp 253–264.

Orle KA, Gates CA, Martin DH, Body BA, Weiss JB (1996): Simultaneous PCR detection of *Haemophilus ducreyi, Treponema pallidum,* and herpes simplex virus type 1 and 2 from genital ulcers. Journal of Clinical Microbiology 43:49–54.

Ross JDC, Smith IW, Elton RA (1993): The epidemiology of herpes simplex type 1 and 2 infection of the genital tract in Edinburgh 1978–1991. Genitourinary Medicine 69:381–383.

Skinner GRB, Billstrom MA, Randall S, Ahmad A, Patel S, Davies J, Deane A (1997): A system for isolation, transport and storage of herpes simplex viruses. Journal of Virological Methods 65:1–8.

Slomka MJ (1996): Seroepidemiology and control of genital herpes: The value of type specific antibodies to herpes simplex virus. CDR Review 6:R41-R45.

Tayal SC, Pattman RS (1994): High prevalence of herpes simplex virus type 1 in female anogenital herpes simplex in Newcastle upon Tyne 1983–1992. International Journal of STD AIDS 5:359–361.

Verano L, Michalski FJ (1995): Comparison of a direct antigen enzyme immunoassay, Herpchek, with cell culture for detection of herpes simplex virus from clinical specimens. Journal of Clinical Microbiology 33:1378–1379.

Whitley RJ, Gnann JW (1993): The epidemiology and clinical manifestations of herpes simplex virus infections. In Roizman B, Whitley, Lopez C (eds): "The Human Herpesviruses." New York: Raven Press Ltd., pp 69–105.